Description on anatomy and histology of *Echinometra mathaei* (Echinoidea: Camarodonta: Echinometidae), the Persian Gulf sea urchin

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Abstract

Echinoids were collected at depth of 0-5 m from coastal water of Larak Island, the Persian Gulf in January 2016. Soft tissues of the animal including peristome, peritoneum, gills, podium, ampulla, axial organ, gonads and food canal were taken and fixed in %10 formalin. Five micron sections were stained by hematoxylin and eosin method and studied under light microscope. The anatomy and histology of organs including peristome, peritoneum, gills, podium, ampulla, axial organ, gonads and food canal were investigated. In oral view, peristome, mouth, teeth, gills, spines, and pedicellariae were the six major compartments. Aboral view was seen as a round shape consisted of tube feet, spines, pedicellariae, madreporite, genital plate, ocular plate and anus. In both oral and aboral view seven types of spines and five types of pedicellariae were reported. Histological evaluation showed the organization of their layers and cells. In this study some anatomical and histological features of the Persian Gulf *Echinometra mathaei* were characterized. The data presented in this paper could be illuminating the path for marine biologists, anatomists, and histologists to use this creature in their desired research areas, basic or industrial approaches.

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Introduction

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In spite of significant value of The Persian Gulf ecosystems in terms of biodiversity, a few studies were locally conducted on marine animals of its environment. Up to now, no study was documented regarding anatomy, histology, and cell morphology of the Persian Gulf sea urchin, *E. mathaei.*

The sea Urchins belong to the phylum of Echinodermata, a large group of marine basal deuterostomes with 7000 species that originated from Cambrian period.

Although sea urchin was named by Klein as Echinoderm in 1734 but Linea classified this creature in invertebrates (Molluscs). Lamark in 1801 located these creatures in radiata group and classified them along with sea stars and sea cucumber in the phylum of Echinodermata (Prouho 1887). The first species of the Persian Gulf Echinoidea was reported by Seba in 1758 (Mortensen & Gislen 1940).

Up to now, about 8000 species of sea urchins (Echinometra genus) have been reported in oceans as common marine invertebrates. Echinometra genus consists of seven species including *E. insularis*, *E. lucunter*, *E. l. polypora*, *E. mathaei*, *E. m. oblonga*, *E. vanbrunti*, and *E. viridis* (Kroh & Mooi 2013). They live in a wide variety of depth from coastal waters to deep regions of West Indian Ocean to Hawaii territory (Clark & Rowe, 1971; Kroh & Mooi, 2013).

The sea urchins groups have been founded in the middle or the lowest area of marine coral reefs near the coastal areas, walk away during night and feed from alga, plants, and dead creatures (Hyman, 1995).

The external bodies of sea urchin are classified in two regular and irregular groups. Regular one has pentagon include sea urchin and the irregulars include sand dollar and heart like morphology. The color in sea urchins is varied from black, red, and brown to purple and light pink (Uexkull, 1896, 1900 & 1907).

The body shape in sea urchin is spherical surrounded by sharp spines and hundreds of tube feet provided them the ability to move on rock and soft surfaces. Their mouth composed of five teeth surrounded by a soft tissue (Amarowicz, Synowiecki & Shahidi 2012).

The coelomic cavity is encompassing gonads, aquatic vascular system, and the gut that are suspended in the coelomic fluid. Coelomic fluid contains different kinds of cell populations. The entity of this fluid is similar to sea water, but has lower salinity and also includes some proteins (Smith, Ghosh, Buckley, Clow, Dheilly, Haug, Henson, Li, Lun, Majeske, & Matranga 2010).

Among seven reported species of Persian Gulf and Oman Sea Echinoidea, *E. Mathaei* is more frequent than the others and lived in coastal water reefs and rocks (Khaleghi & Owfi, 2010; Mahdavi, Haghighat Khazaei, & Karamzadeh 2008). It was first reported by Blainville in 1825 (Mortensen, 1943; Nisiyama 1966).

As there was no comprehensive study concerning characterization of the Persian Gulf Sea urchin, *E. mathaei*, this study was aimed to investigate the anatomy and histology of some of its compartments.

Materials and Methods

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Sample collection

Five specimens of *E. mathaei* ranged from 55 to 70 g were collected manually at depth of 0 to 5 m from coastal waters of Larak island, The Persian Gulf (26°51’12” N 56°21’20” E) in January 2016. The specimens were kept alive and transferred to the laboratory. The specimens were maintained in a flow-through aquarium and were acclimatized at least for two weeks prior to experiments. Genus and species of the collected specimens were determined based on morphological determinants described by Price (1983 and 1986).

Anatomy

To study of the anatomy of the specimen, its general characteristics including shape, size, color, spines, tube feet, and shell were investigated.

External view was classified to oral and aboral poles. In oral view, mouth and its peripherals (i.e. teeth, peristome, and pedicellariae) were investigated. In aboral view, anus, madreporite, gonopores, and genital plate and foramen were studied.

Before access to internal organs, to ethically manipulation of the specimens, they incubated on ice for 45 min. In internal view, coelomic cavity, digestive system, Aristotle’s lantern, ampulla, gonads, epithelium (Peritoneum), and axial organ were evaluated. All of observations were macroscopically documented by a digital camera (canon kiss x3).

Histology

Internal organs including epithelium, tube feet, ampulla, axial organ, digestive system, peristome, and gonads were removed on ice and fixed in buffered formal (10%, pH = 7) (Sigma Co. Germany) . After 48 h, the tissue samples were processed in an automated tissue processor and embedded in paraffin wax. The sections at 4–5 µm thick were prepared and stained with hematoxylin and eosin (H&E) stain.

Results

Taxonomy of *E. mathaei*

The position of this genus is presented in Table 1.

Table 1. Systematics of *E. mathaei*

Phylum	class	order	Family	Genus	species
Echinodermata	Echinoidea	Echinoida	Echinometridae	Echinometra	mathaei

Anatomy

External anatomy was classified to oral and aboral views. In general view, peristome, tube feet, spines, pedicellariae, and gills were seen. *E. mathaei* was spherical in shape and had a dark violet or brown color. The size of our samples was varied from 4 to 8 cm. The average weight of the samples was 65 ± 5 g.

Oral view

In oral view, peristome, mouth, teeth, and gills were the four major compartments (Fig.1).

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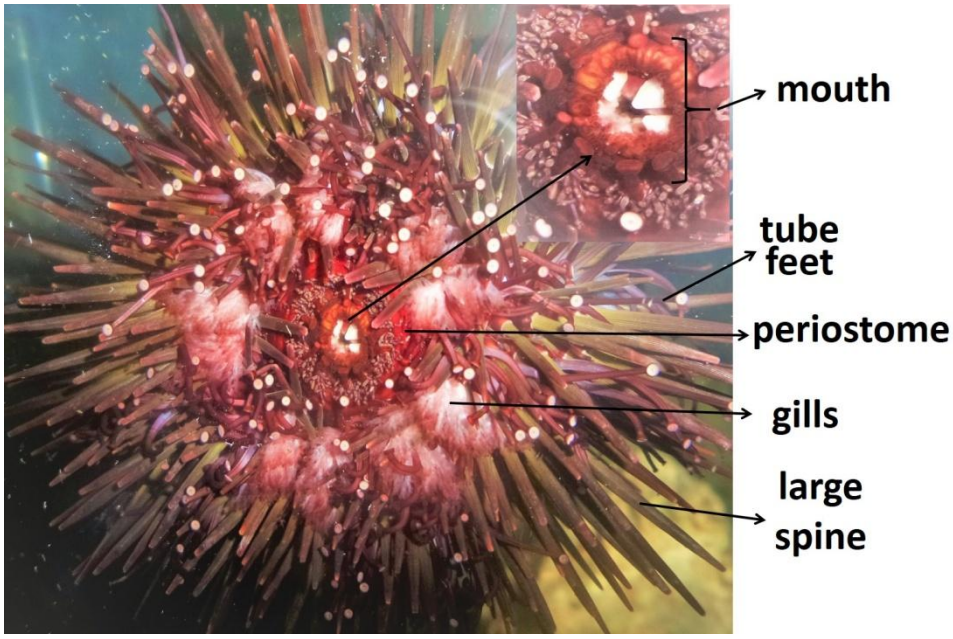


Figure 1. Oral view of the Persian Gulf sea urchin, *E. mathaei*. Image demonstrates elements of external morphology including mouth, tube feet, spines and gills.

Peristome and mouth

Peristome consisted of a peripheral layer in red color (Fig. 2A) that was free of spines and a few small pedicellariae located on its surface (Fig. 2A). These pedicellariae were shown as small or medium size rods with a thicker head resembling a closed fork (Fig. 2A). The inner dark brown layer was adjacent to the mouth and heavily carpeted with small pedicellariae (Fig. 2B). Five pairs of small tube feet were located in circle line in periphery of inner layer of the peristome (Fig. 2A). Mouth was located in central lower oral view consisted of five teeth (Fig. 2B) which were surrounded by a soft layer called as lip (Fig. 2B). Five diaphragms like tissues in pink color were located in the below of each tooth (Fig. 2B). In semi- or complete closed mouth view, an array of many overlapped conical tissues was seen in pink to red color that was surrounded by a circular dark red line (Fig. 2B). Since, 5 pairs of small thick tube feet were located just in the periphery of the mouth, it was speculated that these were interfering to tightly sticking to the desired food to immobilizing it to be dispersed by the teeth. The origin of this kind of tube feet and their mode of action can be a challenging issue that may induce an interest to future studies. According to our observation, the five teeth were capable to rotate in different directions. This flexibility was arisen from the flexibility of connective tissue in peristome. Furthermore, we observed that the peristome can elongated centrally to cover the teeth and this may lead to close the mouth cavity.

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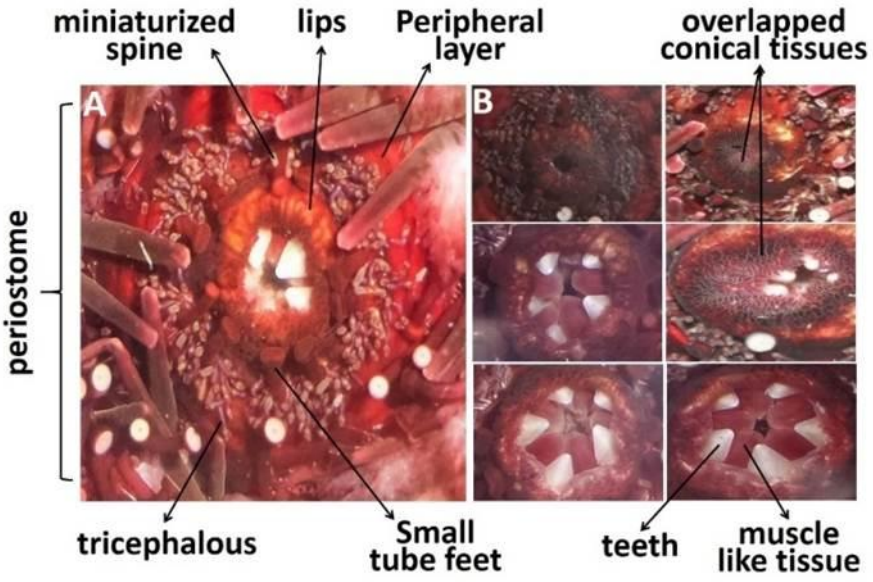


Figure 2. Macroscopic demonstration of peristome. (A) Peristome was a region around the mouth of sea urchin carpeted with spines, tube feet, and pedicellariae. There were five pairs of small tube feet in peristome. (B) The mouth had five teeth surrounded by five diaphragms like tissues.

Spine

The large spines were conical rods that small parallel grooves conducted from their base to the head. The head seemed to be smaller and thinner than the pink body (Fig. 3). The body of spine was originated from a flat red or brown base. A white spotted margin between base and rod was shown under normal or artificial light. The colors of large spines under flash light were green to brown from the base to the head whereas in normal light the spine color was dark brown.

All spines were conical dark brown rods that originated from a red-brown flat base. Some miniaturized spines were located between tiny, mini and small size spines in oral and aboral views (Fig. 3).

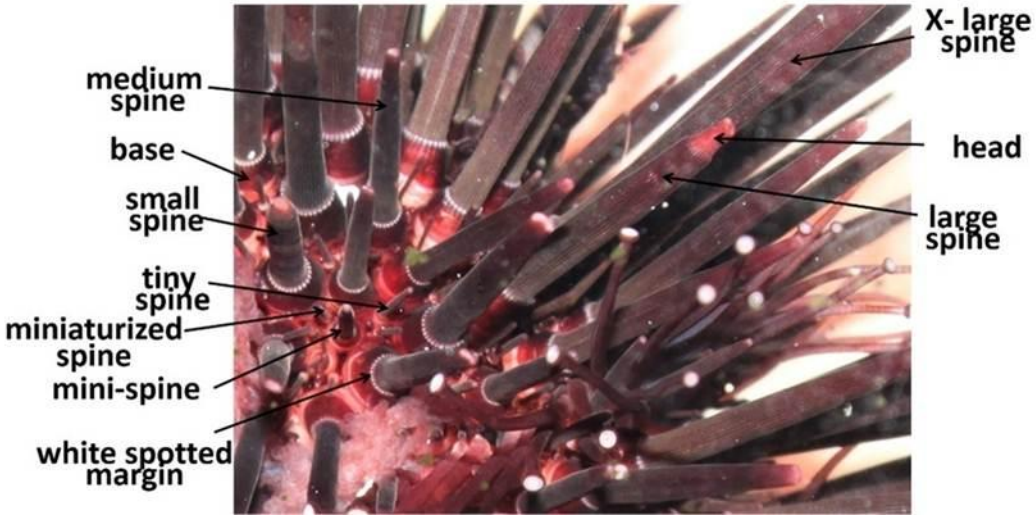


Figure 3. Different kinds of spines in *E. mathaei*. Tiny, mini, medium, large, and X-large spines were located in oral and aboral views.

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Pedicellariae

There were five kinds of pedicellariae including small tridentate, large tridentate, trifoliate (triphyllous), tricephalous, and globiferous ones. It is the first report for observation of a different kind of pedicellariae designated by our team as tricephalous.

The pedicellariae were distributed between the spines and tube feet in oral and aboral surfaces (Fig. 4 and 7).

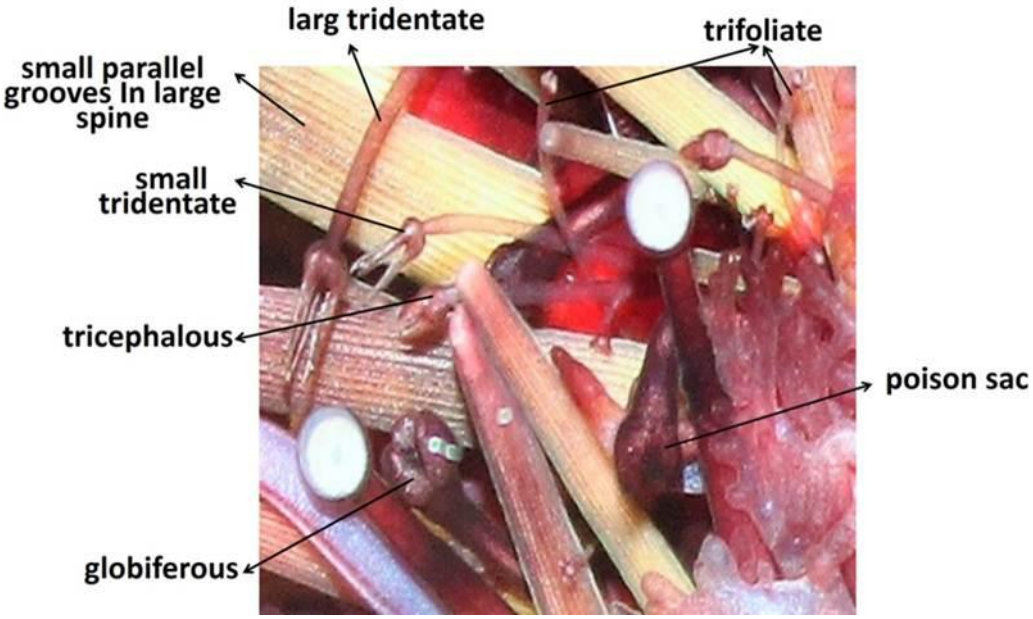


Figure 4. Different kinds of pedicellariae in oral view of *E. mathaei*.

Tube feet

Three kinds of tube feet were seen. The two of them were large in size and other type was very small. The two large ones were distributed in oral and aboral views in five rows that were classified to two types including oral and aboral tube feet.

Oral tube foot was consisted of a small cylindrical tube that terminated toward a larger discus head. It was white with a small central foramen. A small bilateral groove was also conducted from shell to head. Aboral tube foot had a thinner cylindrical tube that terminated toward a smaller discus head. It had a similar bilateral groove too.

Small tube feet were located in the periphery of mouth between pedicellariae. Their shapes were the same as large one except for its light red discus head and dark brown dwarf thick body. The numbers of small tube feet were 10 feet that located in pairs in a circle external line in reference to the mouth cavity (Fig. 1, 4, and 5).

Gills

Five pairs of gills resembling undulating white mass were located in the periphery of peristome. Each of them had several main branches that accessory branches were originated from the main one and protruded toward the surface (Fig.1 and 5).

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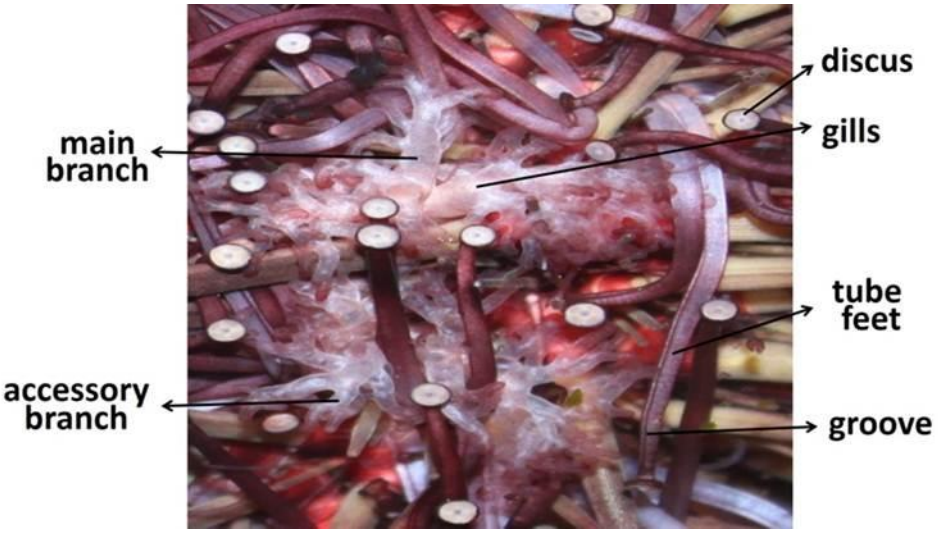
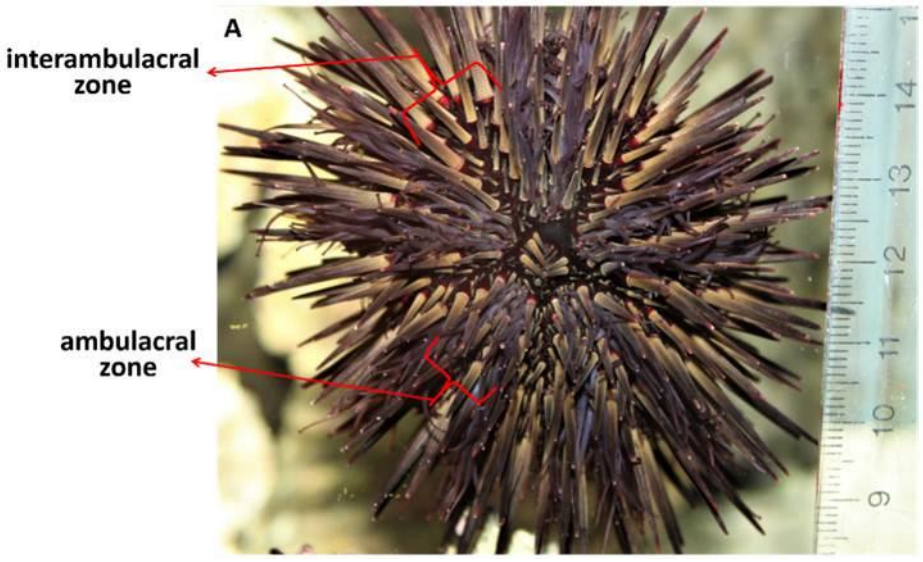


Figure 5. The anatomical characterization of the gills. They located in oral view in five pairs adjacent to the tube feet and spines. They were glassier white appearance with tiny walls.

Aboral view

Aboral view was seen as a round shape consisted of tube feet, all kinds of aforementioned spines, pedicellariae, madreporite, genital plate, ocular plate and anus (Fig. 6A).

In this view, anus was surrounded by a few series of small spines (Fig. 6A, B). Tube feet were exposed in five radial rows in pairs (Fig. 6A). Large spines were distributed in the surface in five radial pairs in adjacent to tube feet rows (Fig. 6B). Medium, small, mini, and tinny size spines were located in the center and periphery of anus too. The central part of aboral view consisted of periproct plate that carpeted by mini and tiny spines. Anus was located in the center of periproct surrounded by tiny and mini spines. Five Ocular and five genital plates as a triangle plates were located in alternate and a pore designated ocular and genital pore were seen in their apical parts (Fig. 6B, 8, and 9).



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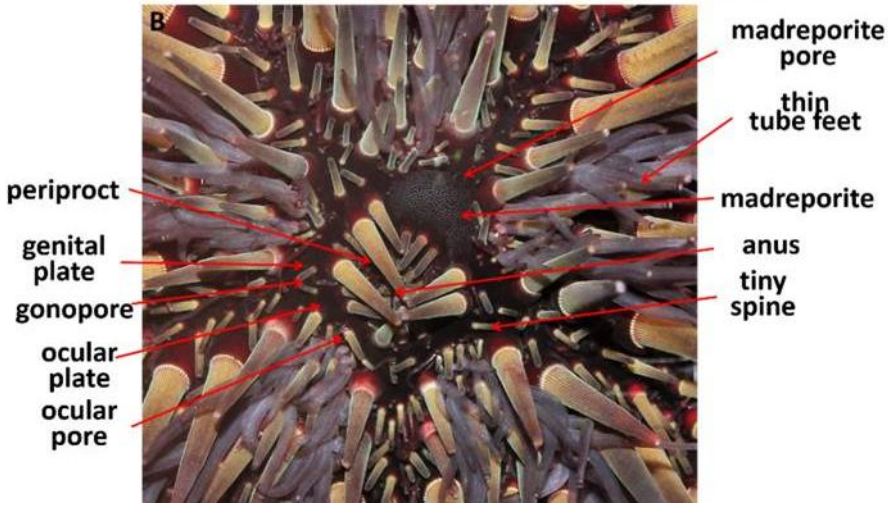


Figure 6. Aboral view of *E. mathaei*. A. Ten meridional rows of tube feet, or podia, expand between the oral and aboral poles. The ten rows of tube feet were decorated into five ambulacra including two rows of tube feet each. The ambulacra were separated by zones without tube feet, known as inter-ambulacral zone. B. The periproct was a small region at the aboral pole circumfluent the anus.

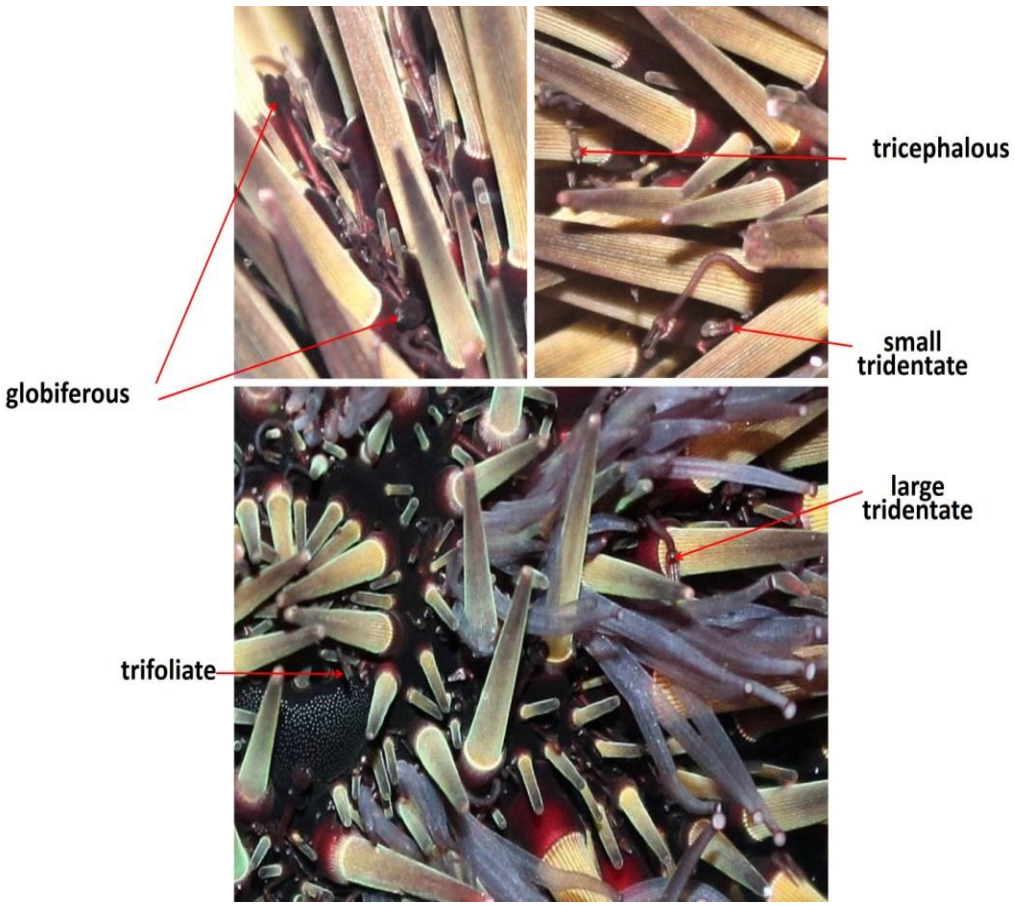


Figure 7. Demonstration of all kind of pedicellariae in aboral view.

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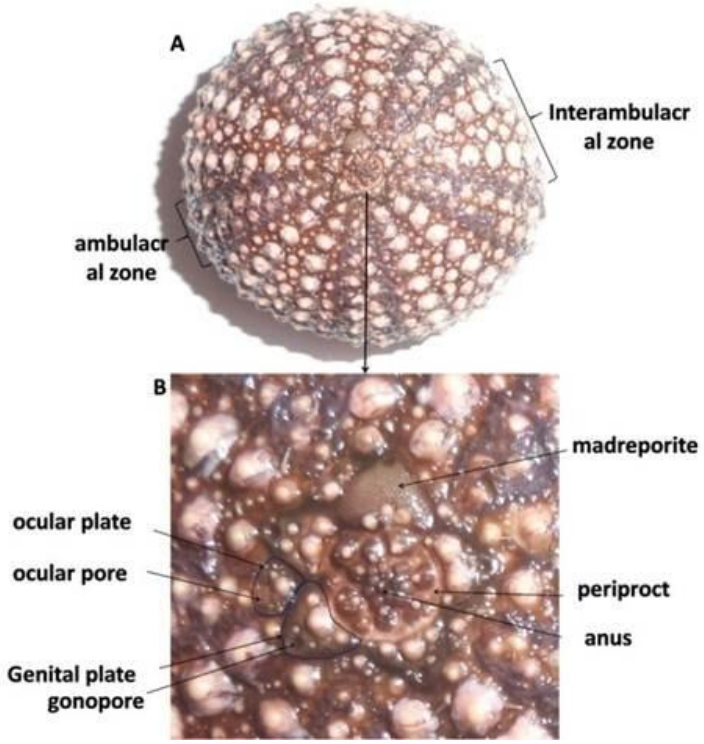


Figure 8. Demonstration of central part of aboral view in *E. mathaei*.

Test

The test was seen as an oval shaped light green hemisphere. The pores corresponding to a tube foot located on test as four pairs and in some cases five pairs that elongated in a semi-circle line between two spines. One of each pore in alternate between the tubercle of two spines had larger size (Fig. 9).

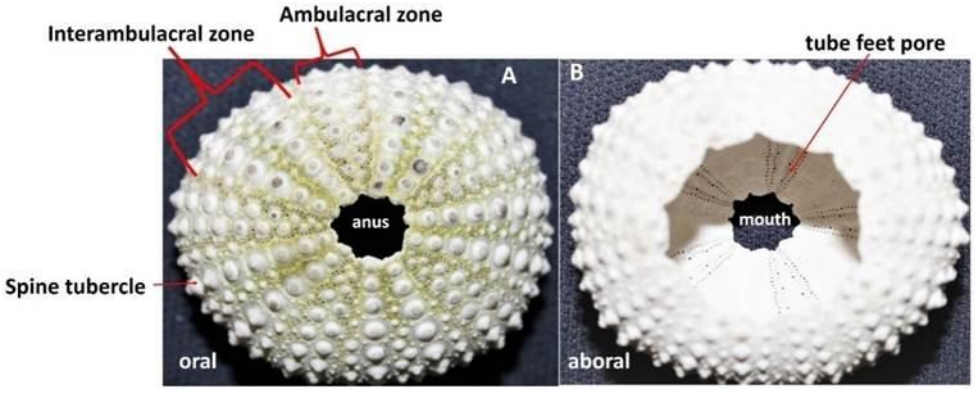


Figure 9. Oral and aboral surfaces of the test in *E. mathaei*.

Internal anatomy

In internal view, Aristotle's lantern, peritoneal membrane and cavity, axial organ, water vascular system, gonad, and digestive system were characterized.

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Digestive system

Digestive system consisted of five main parts: the mouth, esophagus, stomach, intestine and anus (Fig.10). The mouth opened into an esophagus that passed through the center of Aristotle's lantern. The esophagus twisted and turned toward the perimeter and expanded toward stomach. The stomach made a circle inside of the test on the floor of the peri-visceral coelom. The intestine was a vast bag-like shape. Digestive system was attached by mesenteries on the walls of the test (Fig. 10).

The nervous system

The nervous system had a simple plan but it is difficult to observe the neural ring in dissections. There was a large nerve ring surrounding the mouth inside the lantern. Five nerves radiate followed the radial canals of the water vascular system that was originated from the nerve ring (Fig. 10).

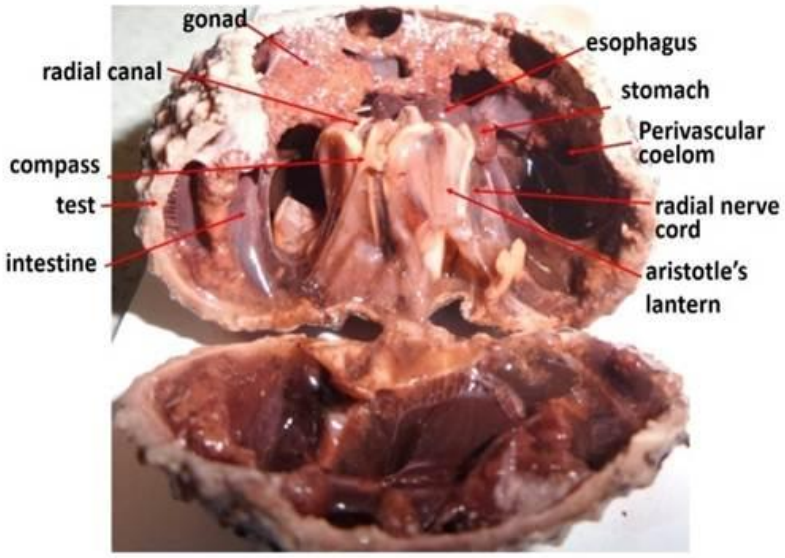


Figure 10. Demonstration of the digestive and nervous systems. Internal organs were located in peritoneal cavity that dissected from other organ by peritoneal membrane. The gonads were surrounded by this membrane. Esophagus was originated from aboral end of the lantern and conducted to stomach. Intestine was shown as vesiculated organs that located in peritoneal cavity and fastened to internal skeleton by several ligaments.

Peritoneum (Peritoneal membrane)

Peritoneum was adjacent to all internal organs, including gonads, digestive system and Aristotle lanterns, and was filled with a liquid called the coelomic fluid. The outer and inner layers of peritoneum were adjacent to the internal skeleton and the coelomic fluid, respectively (Fig. 11, 12).

Aristotle's lantern

Aristotle's lantern was visualized after removing the remainder of the digestive system. Aristotle's lantern was a complex integrated structure made up of multiple hard and soft tissues. This organ was located at the center of the test, on the one hand to the mouth under the peristome and on the other hand to the esophagus surrounding the pharynx. The lantern was surrounded by the peripharyngeal peritoneum. The peripharyngeal peritoneum formed a dental sac around each tooth. Aristotle's lantern was composed of five

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ossicles and related muscles within each ones; these largest bone-like structures resembled a pyramid. The pyramids can act as ‘jaws’, in the sense that they support the teeth. The five pyramids kept up five fragments called rotulas that attached to them by protractor and retractor muscles which provided extension and flexion of the lantern (Fig. 11).

A bony appendix designated ‘perignathic girdle’ was elongated from test to internal space (Fig.11). The girdle joined the test to the lantern out of the muscle groups. The lantern included four different groups of muscles including protractors, retractors, interpyramidals and compass elevators. Protractor muscles were lengthening from the aboral surface of the pyramids to the pergnathic girdle. Retractor muscles were extended from the internal oral pole of the pyramid to the auricles (Fig. 11, 12).

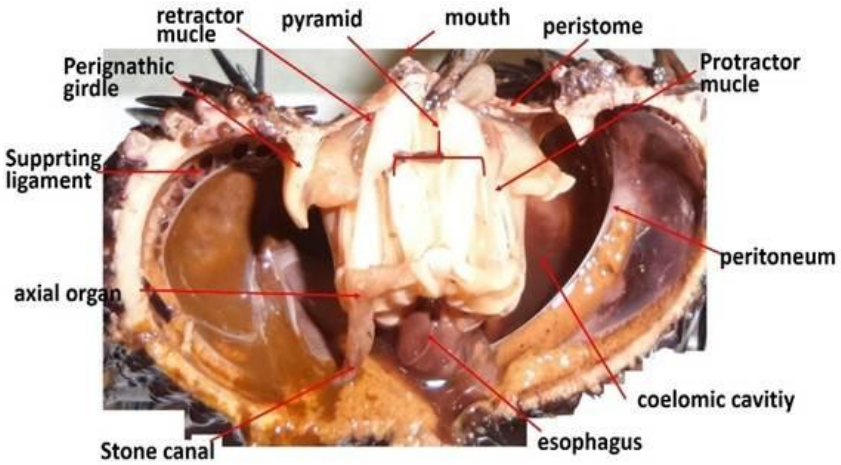


Figure 11. Anatomical demonstrations of the Arisototle’s lantern and other internal organs. The lantern was elongated from oral to aboral end. In this view, it conducted to a pyramidal section that five teeth are protruded from. In aboral view, five curved forks were elongated from lateral body to ring canal. Five radial canals were located between those curved forks surrounded by peripharengial coelom. Reproductive system as a plenty of yellow-golden gonads is located in the periphery of the cavity that ended to five gonopores in aboral view.

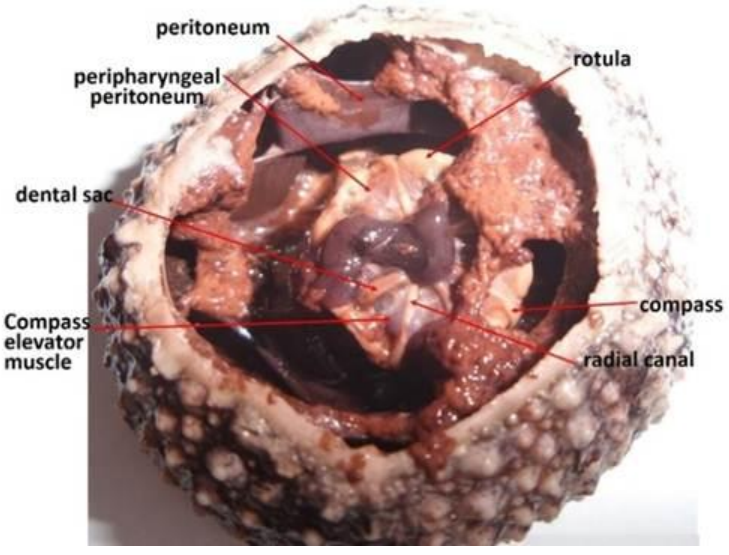


Figure 12. Compass elevator muscles were in periphery of lantern used for extension of the teeth.

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Gonads

E. mathaei had five gonads in the perivascular coelom. Each gonad opened at a gonopore lying in one of the genital plates in the periphery of anus in aboral pole. The gonads were arranged around the periphery of the aboral part of the coelom. Each gonad was located at the center of each interambulacral region surrounding by a transparent peritoneum that binded to the inner walls of the test by several thin supporting ligaments (Fig. 13).

Water vascular system

The water-vascular system started from madreporite through the body (Fig. 6). The madreporite directed to stone canal that was linked into an axial organ, resembling an oval shape sac. The stone canal was a tiny, short, and clear tube that came from one side of the madreporite and opens into the ring canal or water ring (Fig. 13). The ring canal was encircling the mouth which extended to five radial canals. Each of five radial canals made its way to an ambulacrum on the test and then led to the ampulla from which the podia (tube feet) were originated (Fig. 13).

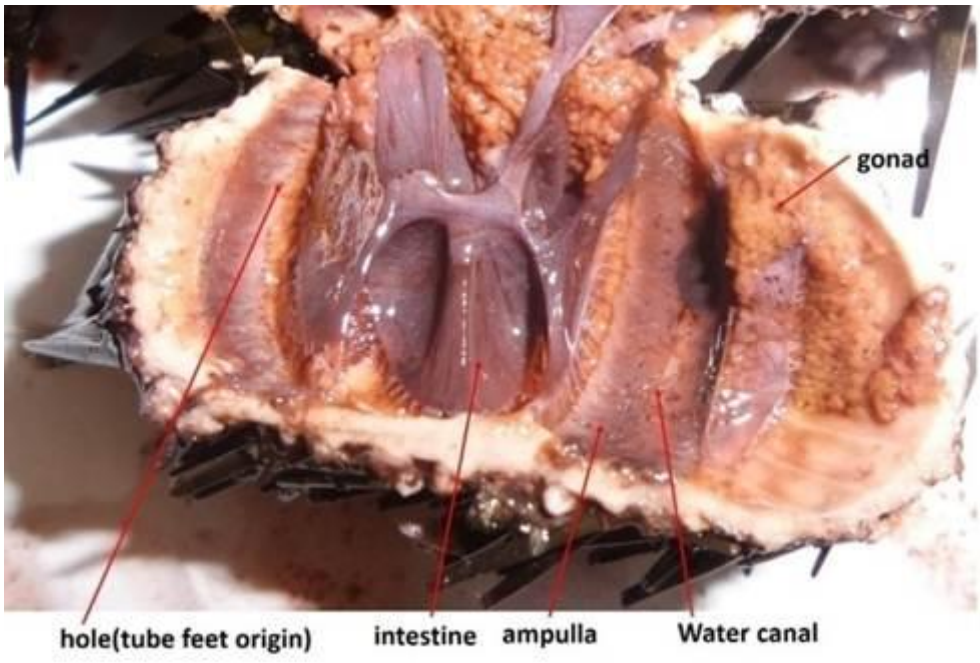


Figure 13. Demonstration of water vascular system. Water vascular system was originated from madreporite and ended to tube feet. Axial organ was originated from the lower part of madreporite that connected to ring canal. Five radial canals was branched from ring canal that elongated to water canal from which paired ampullae were originated from and protruded as tube feet toward external environment.

Histology

Peristome

Peristome is composed of two main layers including a surface dense multi-layered called 'lip' and connective tissue inner layer. The lips contain medium size cells with highly basophilic dense nucleus and moderately eosinophilic cytoplasm (Fig. 14).

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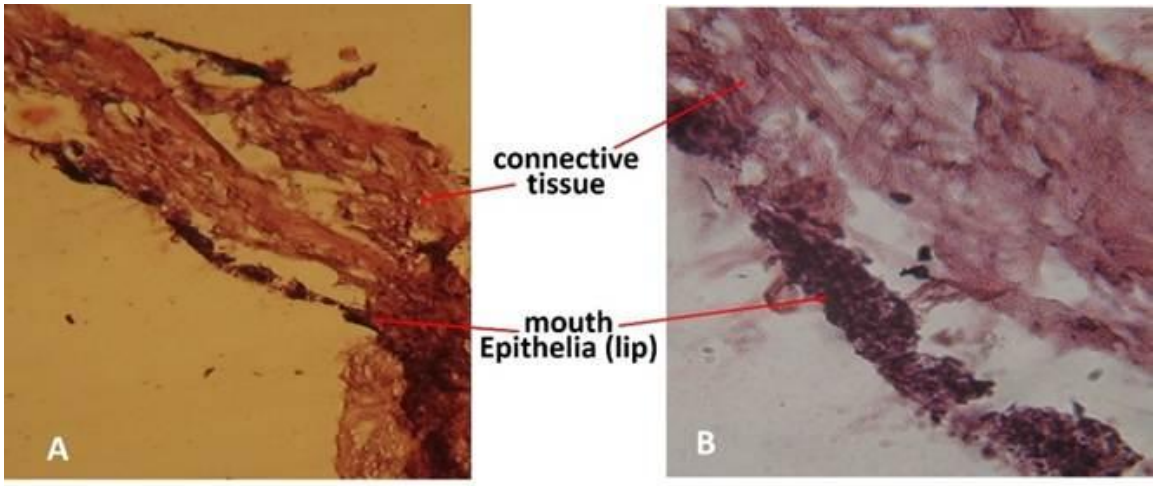


Figure 14. Histological evaluation of the peristome.

Peritoneal (coelomic) membrane

The outer layer of peritoneum was composed of parallel units that were arranged side by side. In each unit, a gigantic cell with a strongly basophilic nucleus was observed. Each unit consists of two kinds of fibroblast like cells with spindly or highly extended nucleus and highly eosinophilic cytoplasm and also two kinds of globular cells with circular dense or transparent nucleolus. Highly dense filamentous structures that elongated along with internal epithelia were seen. Epithelial cells were located on internal side of the peritoneum. In the upper side of epithelial layer, different cells were attached to each other in a parallel form and seemed to be extruding toward the coelomic cavity. These cells were similar to some kind of coelomocytes (Fig.15).

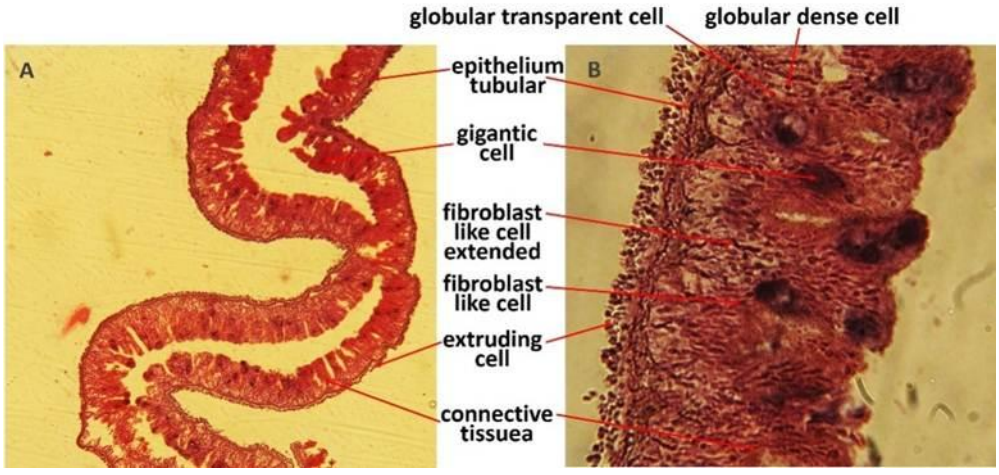


Figure 15. Histological demonstration of coelomic membrane. A. section demonstrated the array of units (4X). B. Different cell layers of the peritoneal membrane (10X).

Gills

In the cross section of gill, main and accessory brunches were observed. Lumen's wall was figured by two layers of cells which surrounded the inner connective tissue layer (Fig.16).

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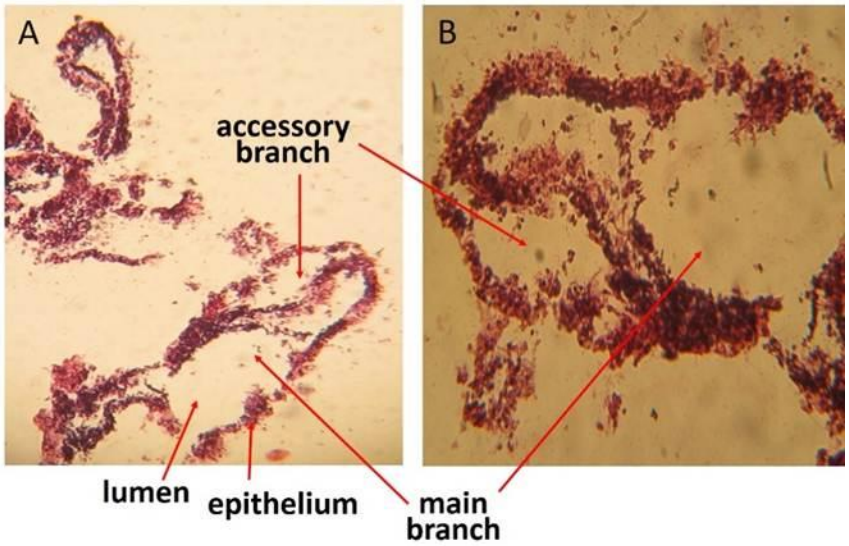


Figure 16. General histology of the gills. A. 4X. B. 10X

Podium (tube foot)

In cross section of a tube foot from lumen to external layer, the cell layers included a basophilic epithelium layer, highly eosinophilic elongated muscles ordered like flagella bundles, a moderately eosinophilic connective tissue, a compact layer bearing neural cell layer with highly basophilic nucleus and highly eosinophilic cytoplasm, and finally several arrows of epithelial cells (Fig.17).

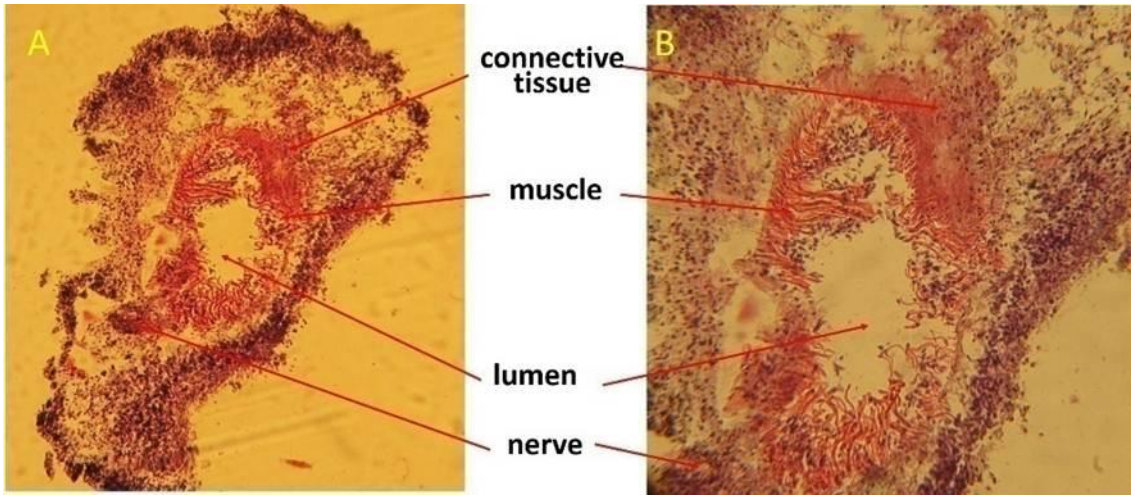


Figure17. Histology of tube feet. A. 4X magnification, B. 40X magnification.

Ampulla

The histology of ampulla was just like tube feet and from internal layer of lumen to external layer, was consisted of a larger epithelial layer which was composed of small cells with extreme basophilic nucleus and a small volume cytoplasm. There was an orange fibrous skeletal muscle cells under the epithelial layer that down-warded into a pink connective tissue layer. There was a thick layer of parenchymal cells under the connective tissue which had a moderately basophilic nucleus and a little eosinophilic cytoplasm ended to external epithelial cells (Fig.18).

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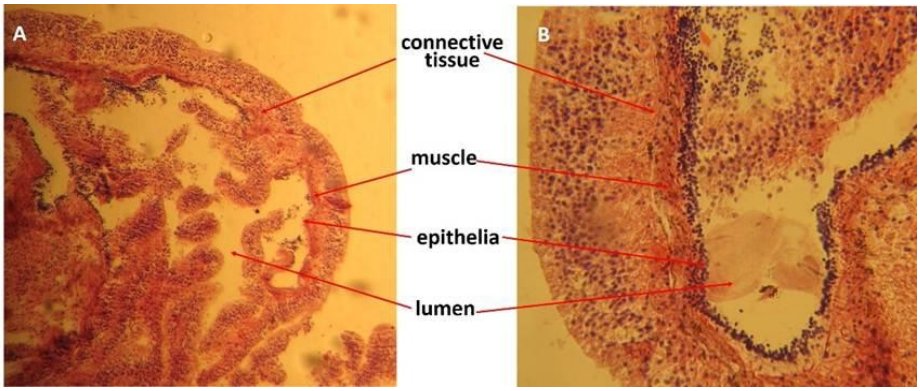


Figure 18. Histology of ampoule. A. 4X magnification of an ampoule indicating a multi-lumen organization. B. 40X magnification of the ampoule showing similar histology with tube feet.

Axial organ

General appearance of axial organ at 4X was similar to a spongy tissue containing many small and large holes. These compartments approximately resembled the human kidney composed of proximal and distal tubes. A tiny dense layer containing epithelial cells was seen as peripheral layer. From surface to depth, proximal and distal tubules were coordinated. N/C ratio in parenchyma and tubular cells were small whereas the marginal clusters had a high nuclear–cytoplasmic ratio. Several compact clusters of basophilic cells were seen as marginal disjointed layer that located between the boundaries of these tubules (Fig.19).

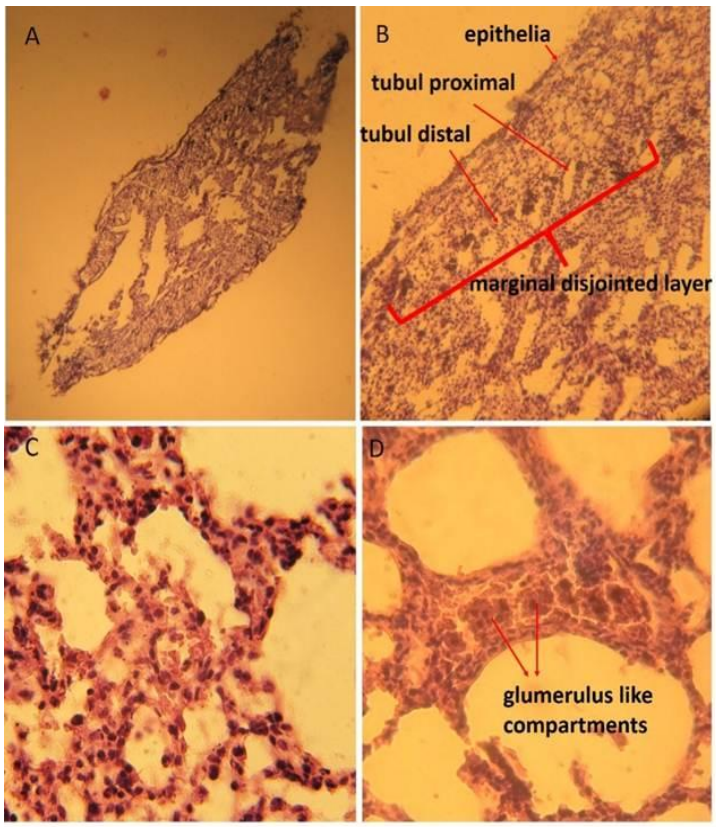


Figure 19. Histology of axial organ. A. General structure of the axial organ (4X). B. A magnified section of axial organ showing epithelial layer, proximal and distal tubules. C. Proximal and distal tubules carpeted with tubular epithelia. The parenchyma cells were seen within the tubules (100X). D. A cross section of tubules containing several glumerulus like compartments (10X).

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Gonads

Each gonad was composed of several separated units in which two kinds of cells were observed. Peripheral cells as a single layer had highly basophilic nucleus with high N/C ratio. Inner cell layers below the peripheral cells resembled fatty tissue with a hyaline appearance. Reproductive cells were located centrally and their N/C ratio was significantly high (Fig. 20).

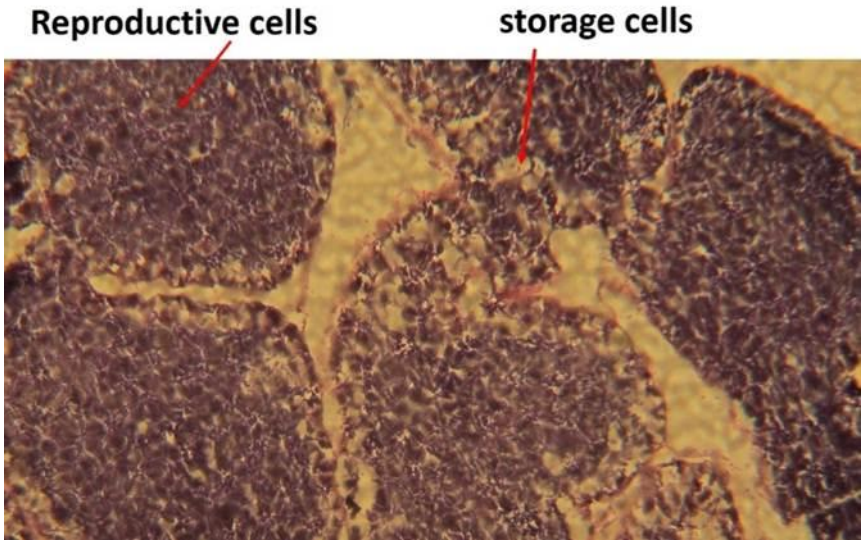


Figure 20. Histology of the gonads in *E. mathaei* (40X).

Esophagus

Esophagus was composed of mucus layer, submucusal layer, connective tissue, and muscle layer. Mucusal tissue composed of several cell with highly dense basophilic nucleus distributed across a pink connective tissue. From this layer, in a regular order, parallel appendices directed into the lumen. The clusters were beside each other and separated by open space. Each appendix as a cellular cluster contained many cells that compacted side by side. These cells had extreme basophilic nucleus and extreme eosinophilic cytoplasm. Submucusal layer was not clearly distinguishable. The inner layers contained connective and muscular tissues (Fig. 21).

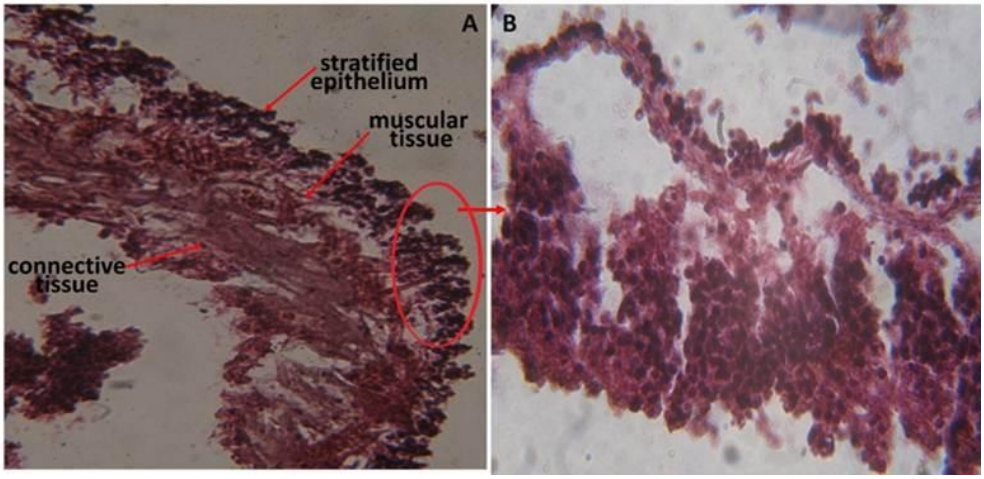


Figure 21. Histology of esophagus. A. 10X. B. 40X

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Stomach

Histological organization of stomach was similar to esophagus in exception of cluster appendices in the mucosa resembling parallel columnar villi. Epithelial cells were seen in a surface layer and carpeted the whole surface. Epithelial cell had moderately basophilic nucleus and moderately eosinophilic cytoplasm. The marginal cells of submocusal layer was apparently separated the mucosa from submucosal layer. This layer contained fibroblast like and paranchaymal cells with larger dense basophilic nucleus with more eosinophilic extent comparing to the surface layer (Fig. 22).

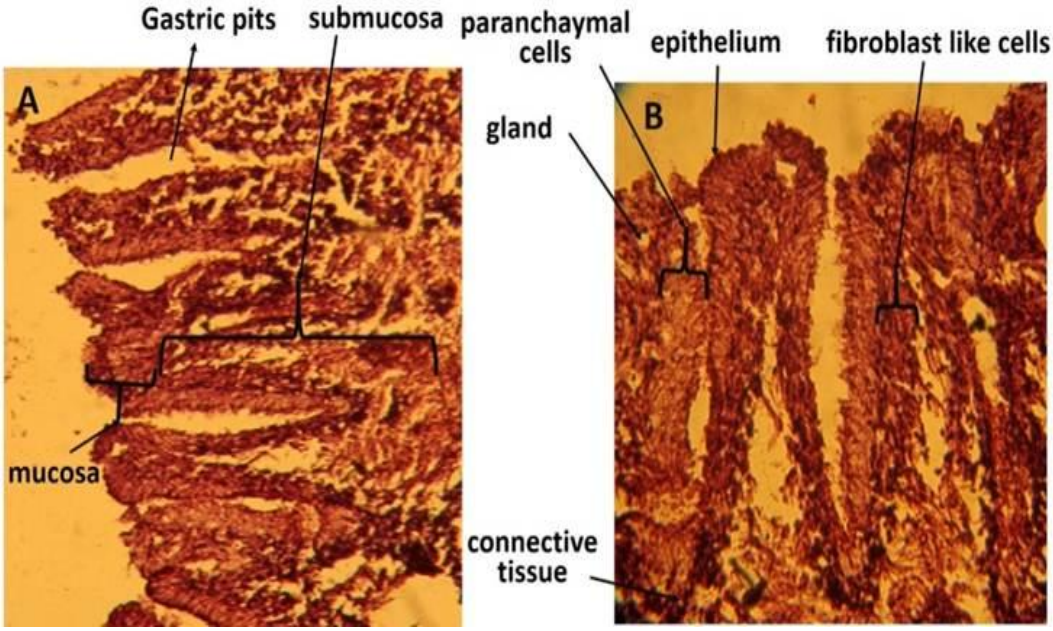


Figure 22. Histology of stomach at 10X magnifications.

Intestines

The general histological organization of intestine was similar to stomach. Lumenal layer composed of columnar narrow villi. Mucosal layer in the surface of each villi contained two kinds of cells. External layer had small cells with dense and slightly basophilic euchromatin nucleus containing several small dense basophilic nucleoli with moderately eosinophilic cytoplasm. This layer was obviously marginated by a marginal cell layer belong to submucosal layer. This layer was composed of large cells containing large highly basophilic dense nucleus with moderately small eosinophilic cytoplasm. The inner layer of submucosa contained fibroblast like cells settled in parallel, in the center of submucosal layer. These cells had spindly moderate basophilic nucleus with large moderate eosinophilic cytoplasm (Fig. 23).

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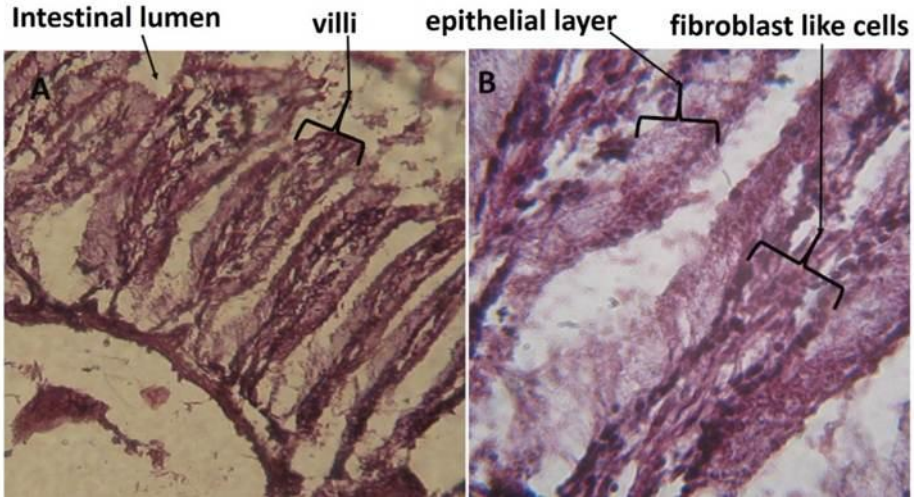


Figure 23. Histology of small intestine. A. 10X, B. 40X.

Discussion

Some incomplete and discrete studies had been performed on characterization of some echinoids like red (*Strongylocentrotus franciscanus*), green (*Strongylocentrotus droebachiensis*) and *Paracentrotus lividus* sea urchin (Amarowicz *et al.* 2012; Arizza, Giaramita, Parrinello, Cammarata & Parrinello 2007) but no study had been comprehensively focused on anatomy, histology, and cytology of *E. mathaei*.

Many years ago, Mortensen and Gislen in 1940 reported 11 echinoid species from the Persian Gulf (Mortensen & Gislen 1940) thus study of the Persian Gulf these echinoid are being necessitated. Accordingly, we tried to characterize the Persian Gulf *E. mathaei* in terms of surface morphology, anatomy and histology of some external and internal organs, and microscopy the coelomocytes regarding their surface and internal morphology.

E. mathaei is displayed by vast variety in form of test and spines, as well as in color and shape (Mortensen 1943; Arakaki, Uehara & Fagoonee 1998). Our specimens are also coincided with these prior morphological explanations for *E. mathaei*.

E. mathaei body has a radial symmetry with body parts occurring in fives. Radial symmetrical creatures have a top and bottom, but do not have a left and right or determined front or back part. Penta-radial symmetry organisms can generally move slowly and easily in any direction (Omni-directional) (Morrissey & Sumich 2012).

E. mathaei like most regular sea urchins has domical shape with one flattened pole. The surface of the body is hard and stiff due to the underlying test. It is also enables a place for the spine of the sea urchin to attach onto (Drozdov, Sharmankina, Zemnukhova & Polyakova 2016).

Our observation demonstrated two types of spines including blunt and sharp tip were seen regarding their ending sharpness. The blunt end ones were pinky and seem to be less hard than others, the function of this interesting tip is not clearly determined. Further histological studies

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needs to uncover the issue. The size and trenchancy of an urchin's spines differ from species to species. The genus Diadema has the longest spines (Yokes & Galil 2006) in comparison to *E. mathaei*. The spines are sharp in some species to defend the sea urchin from the risk of predators. We also observed that the spines can move through any side in any direction. The spines can be acting as the first line of defense in the sea urchins. It is the first report regarding classification of the spines to seven types based on their size. During our observations for at least one year on collected specimens in our aquarium, we proved that the small spines are not in primary stage to be a large one. The other testimonial to confirm the categorization of the spines to seven types in *E. mathaei* was observation of seven kind of spine tubercle in the surface of the test that constantly specified for each kind of spines.

The pedicellariae can act as the biting jaws and are the second line of defense in the sea urchins (Moitoza & Phillips 1979). Four types of pedicellariae are moving toward different direction and recurrently open and close their sharp forks. Our observation is in consistence with study of Moitoza and Phillips in 1979.Globiferous pedicellariae in *E. mathaei* is closely similar to the ones reported by Campbell in 1983 that their function characterized as a poisonous gun (Campbell, 1983). According to this high similarity in morphology, globiferous pedicellariae in *E. mathaei* may contain poisonous materials. This suggestion may be clarified in forthcoming studies (Ghyoot, Ridder & Jangoux 1987).

The mouth is basically a large hole in the center of the oral surface of the test, surrounded by a flexible membrane, Peristome. The Peristome is an important part of the lantern in regular echinoids (Bonasoro, Candia & Wilkie 1995).Our observations were in consistent with other echinoids like *Paracentrotus lividus* in which the teeth and their peripheral layer approximately were similar to *E. mathaei* (Bonasoro *et al.* 1995). Our research on the histology of the Peristome acknowledge the functional versatility of this structure in regular sea-urchins and the functional grandeur of its connective tissue component which has been illustrated to be a general variable collagenous tissue (Wilkie, Candia & Andrietti 1994; Wilkie & Emson 1988).

E. mathaei like other species of the sea urchins has a specialized jaw structure to permit them to feed effectively on algae. This similarity is might due to similar diet for crushing the soft and hard foods.

Kaneko (1975), Cook and Kelly (2007) were stated that *E. mathaei* can feed from algae but this question is claimed that, why these hard teeth are observed in their mouth. We proposed that *E. mathaei* can feed from hard materials that must be investigated in future studies.

Anatomical and histological descriptions of the food canal of regular echinoids were given by Chadwick (1900) and Stott (1955). Even though *E. mathaei* is a common sub tidal species along the world`s oceans, no detailed anatomical and histological studies on its digestive system have been made yet. Boolootian (1964) described the structure and function of the food canal and associated hemal

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canals in *S. intermedius* in some details. From the point of histology, the wall of the food canal is combined of an outer epithelium, layers of circular and longitudinal muscles, a layer of connective tissue of varying thickness, and an inner epithelium commonly implicate of very tall, slender cells (Boolootian,1964). In general, the histology of food canal in *E. mathaei* was the same as Boolootian observations.

Stott (1955) explanations showed that the esophagus wall in *Echinus esculentus*is merely composed of a constant layer of tall, narrow, cylindrical mucous secreting cells. These findings agree with the observations presented here on *E. mathaei*. The inner epithelium of the stomach in *S. intermedius* consists of long columnar cell units which contain small granules. These granules are distributed in the stomach epithelium special whereas in *E. mathaei* no granules were seen.

Histology of stomach in *E. mathaei* was generally was similar to *S. intermedius*. Boolootian's observations in 1964 concerning the histo-morphological complement of the food canal in *S. intermedius* agree with our findings on *E. mathaei*. Few studies have been made on how nutritive substances are being transmitted between the gut and other organs. Farmanearmaian and Pmllivs (1962), Giese, Greenfield, Huang, Farmanfarmaian, Boolootian and Lasker (1959), Greenfield, Giese, Farmanfarmaian, and Boolootian (1958), and Stott (1955) imagine that the coelomic fluid can act as a nutritive pathway although the physiology of the coelomic fluid system and their role in the transport of the nutrients stands unknown yet.

Tube feet are the external appendages of the echinoderm water-vascular system and present a remarkable diversity of forms and functions (Flammang, 1996). Steen in 1965 assumed that the fleshy tubular legs are parts of the hydro-vascular system and play an important role in movement, respiration, and sensation. Lesser, Carleton, Böttger, Barry, and Walker in 2011 confirm that in the green sea urchin (*Strongylocentrotus droebachiensis*), the most distal section of these tube feet contain five ossicles arranged as a light gatherer with its concave surface facing towards the ambient light. This newly described function is in addition to the formerly identified functional roles of tube feet. These anatomical characteristics of the tube feet are consistent with those of other members of Echinodermata family like *P. lividus* and *M. glacialis*. A disc at the distal end of each tubular leg is used to stick and also to receive input senses. Based on our observation, the aboral tube feet randomly move through different directions resembling the sensory organs tried to smell something. Moreover, we observed that the oral tube feet were able to bind themselves to the glass wall of the laboratory aquarium during movement of the sea urchin. Since a muscular layer was seen in histology of tube feet, we assumed that they play an important role in the movement of sea urchins. The role of oral tube feet in the movement are boosted by this fact that the vascular-water system are ended to tube feet and also there is a pore in the center of distal section to work as a vacuum to strong binding of sea urchin to vertical surfaces. The observed differences in anatomy of aboral and oral tube

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feet can be clearly assisted in assumption of their different functions. The tube feet can provide a suitable model for the regeneration of the nerves and muscle tissue in particular as noted by Czerny and Busslingerin 1995.

The gills are proposed to be the respiratory organs for coelomic cavity and also supply the numerous, highly active lantern muscles with oxygen (Burnett, Terwilliger, Carroll, Jorgensen & Scholnick 2002). *E. mathaei* like other echinoderms have a poorly expanded respiratory system (DeFur & McMahon 1984). Reference to our observations, in terms of anatomy, the gills was being arranged in five positions in oral view in the periphery of peristome. Hyaloid appearance of the gills and having many branched tubules were resembled the bronchioles in vertebrate and human lungs to expand the surface for absorbing more oxygen molecules. Histology of the gills was verified the void structural organ. This observation is confirmed the assumed function to adsorbing and transferring the air molecules toward internal organs. Histological studies of this organ in species by Morse and Zardus (1997) showed that the gases exchanged in bronchial epithelium in both main and minor trachea.

The water vascular system in various classes of Echinodermata has approximately similar structural organization. This system arrives to body through the madreporite. It is a sieve-like plate and the number of it is varied in Echinodermata. In *E. mathaei* like other Echinoidea the madreporite possesses many pores, except *Echinocyamus pusillus* that is specific in having only one water-pore (Ziegler, Faber & Bartolomaeus 2009). Axial organ is anatomically located below the madreporite plate in echinoid (Millott & Vevers 1964).

Some limited studies about axial organ had been conducted by Millott and Vevers (1964), Bachmann and Goldschmid (1978), Anteunis, Leclerc, Vial, Brillouet, Luquet, Robineaux and Binaghi (1985), and Welsch and Rehkamper (1987). They were stated that the axial organ has a spongy, glandular figure and it is a community of free cells, connective tissue and cell debris floating in a fluid matrix. They noted that the free cells are identical to those found in the coelom and are able to wander between portions of axial organ and can straightly go across the epithelium whereas our histological observations are not in consistence with the abovementioned studies. We found that interestingly the histology of this organ was highly resembled the kidney in vertebrates as this organ has many tubules similar to proximal and distal tubules figure out as a kidney like organ. According to this similarity, we suggest that this organ may interfere in modulation of water and minerals to providing an iso-osmolar environment for the sea urchin. Moreover, we did not see any coelomocytes in the spongy tissue of this organ.

Peritoneum is generally composed of inner and outer layers that contain some parenchymal and giant cells. The outer layer is composed of units that are squeezed together and consists of fibroblast cells with elongated nucleus and highly eosinophilic cytoplasm. These cells can play a role in strength and elasticity of the peritoneum and prevent its rupture. In each unit, a giant cell with a strongly basophilic nucleus

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was observed. These giant cells are likely to act as stem cells and contribute to the formation of cells in coelomic fluid. This hypothesis derives from the fact that in the inner layer there is an undeniable evidence of the cells leaving the interior peritoneum, which can be seen on a regular basis throughout the inner layer. Meanwhile, similar cells are seen inside the tissue, which have similar morphology to exiting cells. These cells seem to be migrating to the perineum interior layer. Liebman (1950) suggested that peritoneum epithelium is the source of coelomocytes in *Arbacia punctulata*.

Reproductive system in *E. mathaei* contains five yellowish gonads. From the point of histology, two main cells including storage and reproductive cells were observed in consistent to other studies about Echinoidea (Tyler, 1939; Nichols, Bishop & Sime 1985). In Echinoidea the gonads are either ovaries or testes and are different in color.

Conclusion

In this study some anatomical and histological features of the Persian Gulf *E. mathaei* were characterized. The data presented in this paper could be delighted the way to marine biologist, developmental biologist, anatomist, histologist, toxicologist, and systematic biologist to use this creature in their desired research area in line basic or industrial approach.

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Authors' Contributions

F.P performed all experiments and also contributed in writing the manuscript. P.M and K.P.B served as supervisors. D.S contributed as advisor. K.P.B contributed in all experiments and also in writing, revision and redaction of the manuscript.

Conflict of interests

The authors declare that there is no conflict of interest.

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آناتومی و بافت شناسی بخش‌هایی از توتیای دریایی (*Echinometra mathaei*) خلیج فارس

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چکیده

توتیاهای دریایی از عمق ۰-۵ متر از آب‌های ساحلی جزیره لارک خلیج فارس در طی دی ماه ۱۳۹۴ جمع‌آوری شدند. بافت های نرم حیوان شامل پریستوم، صفاق، آبشش، پای لوله‌ای، آمپول، ارگان محوری، گناد و کانال غذایی جدا شدند و در فرمالین ٪ ۱۰ فیکس شدند. بعد از برش قطعات ۵ میکرونی، به روش هماتوکسیلین و ائوزین رنگ آمیزی صورت گرفت و تحت میکروسکوپ نوری مورد مطالعه قرار گرفتند. آناتومی و بافت‌شناسی اندام‌ها شامل پریستوم، صفاق، ابشش، پای لوله‌ای، آمپول، ارگان محوری، گناد و کانال غذایی مورد بررسی قرار گرفت. در سطح دهانی، پریستوم، دهان، دندان‌ها، آبشش‌ها، انواع خارها و انواع پدیسلاریا بخش‌های مهم بودند. در سطح مقابل دهانی که به شکل مدور دیده می‌شود، پاهای لوله ای، خارها، پدیسلاریا، مادرپوریت، صفحه جنسی، صفحه چشمی و مخرج وجود دارد. در هر دو سطح، دهانی و خلفی، هفت نوع خار و پنج نوع پدیسلاریا گزارش شد. در این مطالعه برخی از ویژگی‌های آناتومی و بافت شناسی توتیای دریایی خلیج فارس (*Echinometra mathaei*) مشخص شد. داده‌های ارائه شده در این مقاله می‌تواند راهگشای مطالعات آینده برای زیست‌شناسان دریایی، بیوتکنولوژیست‌ها، آناتومیست‌ها، و متخصصین بافت شناسی جهت استفاده از این موجود در زمینه‌های تحقیقاتی مورد نظر با رویکردهای پایه و صنعتی باشد.

کلمات کلیدی: توتیای دریایی، *Echinometra mathaei*، آناتومی، بافت شناسی، خلیج فارس

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